Studying the Blood-Brain Barrier in 3D

Development

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The blood-brain barrier is a critical biological system that regulates protection and support of the central nervous system, but little is known about how it develops and functions. Using Imaris software, the most powerful and versatile 3D and 4D image analysis solution on the market for researchers in life sciences, researchers from Genetech in San Francisco, California, have made new discoveries about the development and function of the blood-brain barrier.

Figure 1. Filament tracing is shown in green and branch points in orange for the hindbrain of a zebrafish embryo 3 days after fertilization.

The blood-brain barrier forms a seal that limits molecular exchange between vessels in the central nervous system. It lets in molecules that are needed for brain function while getting rid of unwanted molecules. Many neurodegenerative diseases involve disruption of the blood-brain barrier, and drugs that can treat central nervous system diseases must pass through the barrier.
“We believe the pathways defined during development, maturation, and maintenance of the blood-brain barrier will be disrupted in disease,” said Dr. Ryan Watts, who led the research team. “We are laying the foundation for a better understanding of the barrier to advance our understanding of this system in the disease state and also to design drugs that selectively cross this barrier.”

The researchers studied the process of sprouting of central nervous system vessels, known as angiogenesis, and the early stages of barrier formation, known as barriergenesis, in zebrafish modified to have non-functioning DR6 and TROY proteins. DR6 and TROY are both death receptors in the tumor necrosis factor receptor family.

Imaris let the researchers examine the phenotype of the zebrafish in three dimensions and identify changes in vascular density. To do this, they imaged the zebrafish using laser scanning confocal microscopy taking z stacks that were ~100 microns thick and then used the Imaris software to perform volumetric and filament tracing analyses. This information was then used to quantify potential changes in vascular branch density, vessel diameter, vascular volume and total brain volume in the zebrafish forebrain. Dr. Stephen J. Tam, who was part of the research team, says that the image masking combined with filament tracing capabilities of Imaris allowed them to quickly quantify their endpoint data with regards to the volumetric and vessel branching analyses, respectively.

The Imaris results along with other analyses showed that both the DR6 and TROY death receptors modulate angiogenesis and barriergenesis during development of the blood-brain barrier. The researchers obtained similar results in mice studies.

This movie shows the Zebrafish hindbrain from 1.5 to 2 days post fertilization. The arrows show new vessels sprouting into brain.

Figure 2. The head vasculature is shown 2.5 days after fertilization as a raw image (top), the mask without raw data (middle), and the vascular mask applied to hindbrain vasculature (bottom).